

## **Supplemental Material to:**

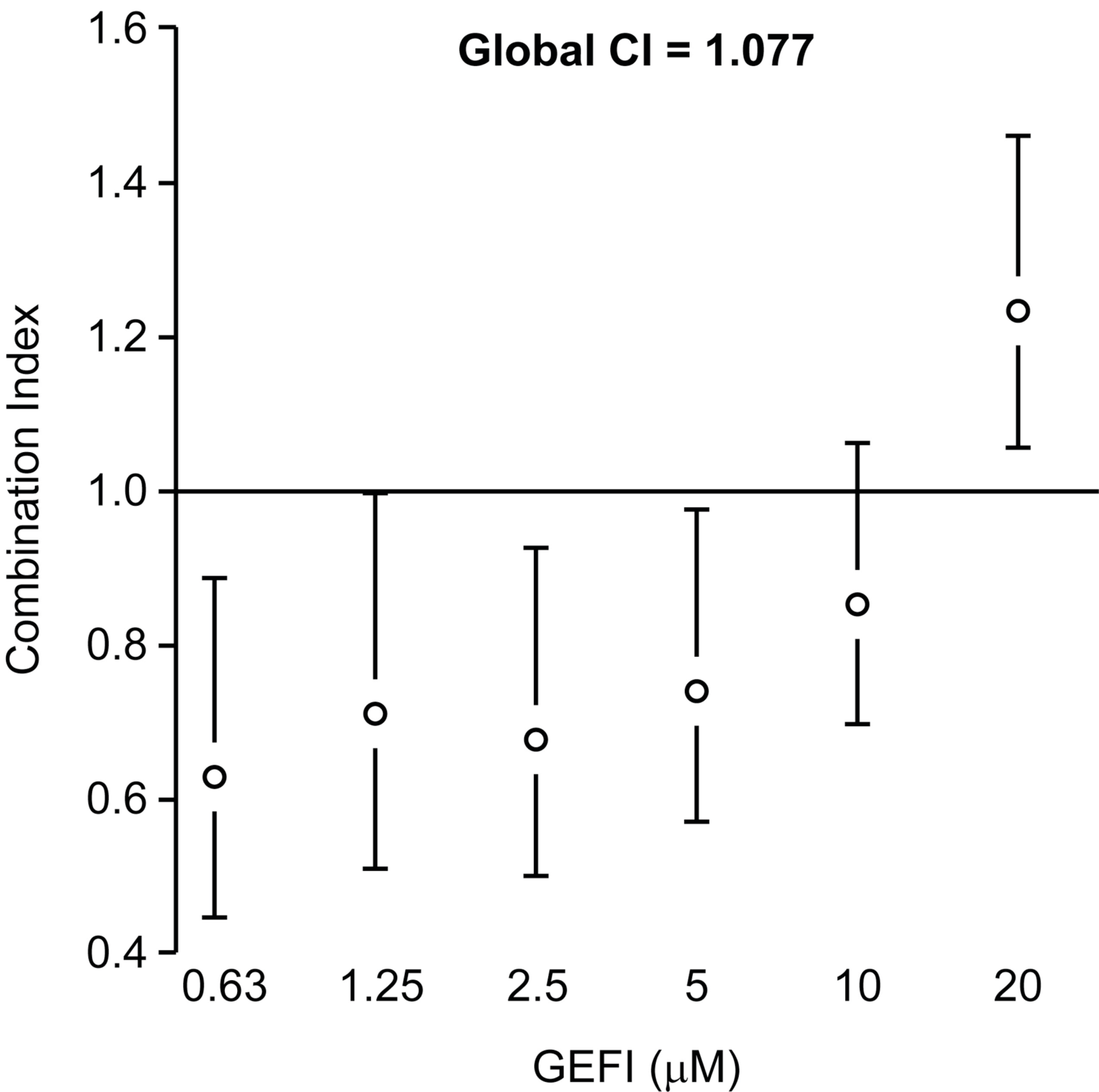
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**Erlotinib antagonizes ABC transporters  
in acute myeloid leukemia**

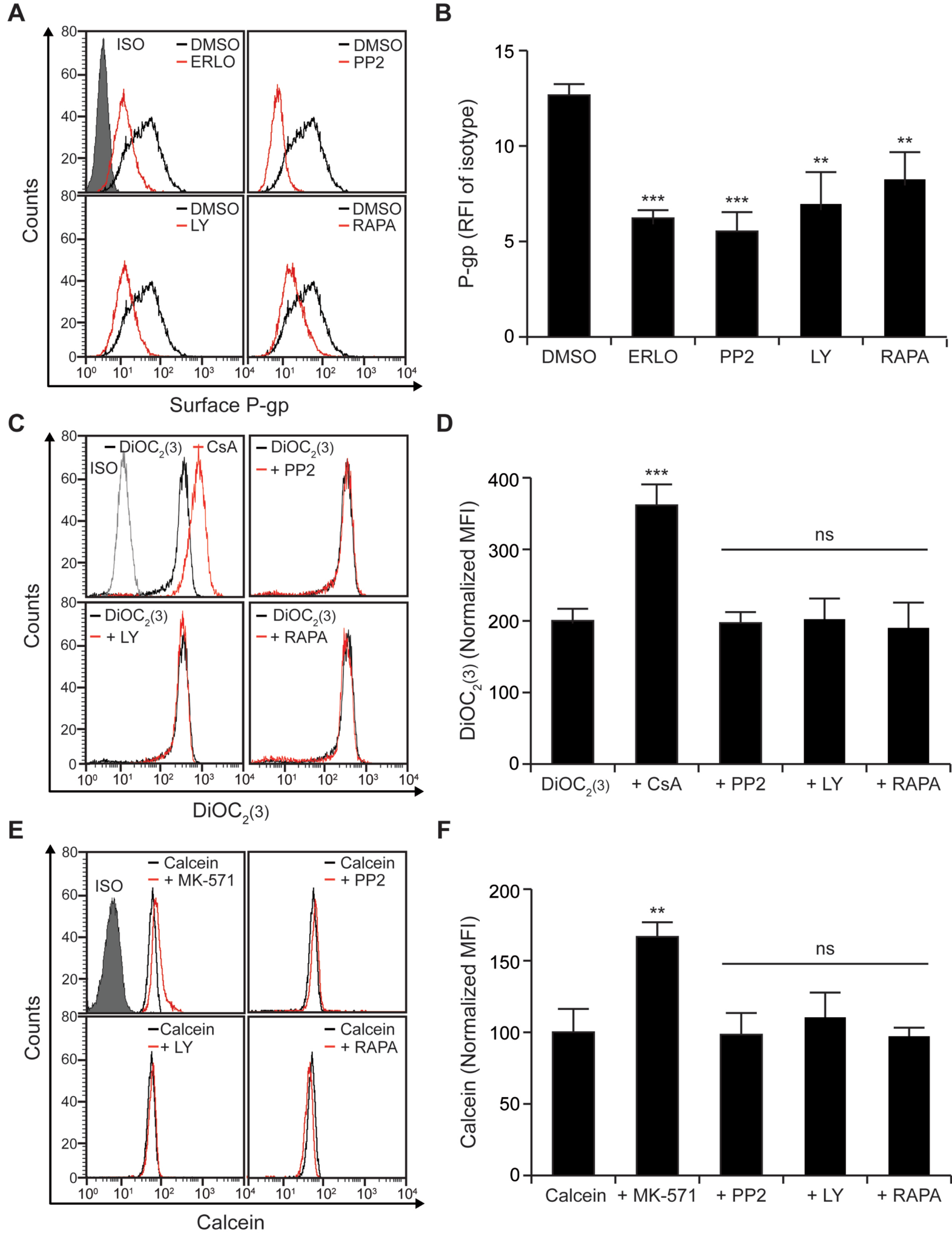
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**<http://dx.doi.org/10.4161/cc.22382>**

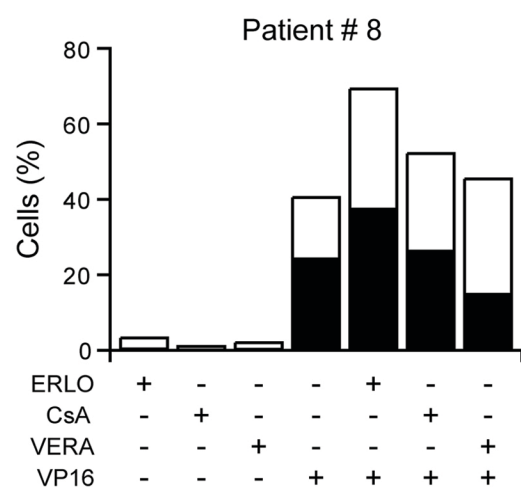
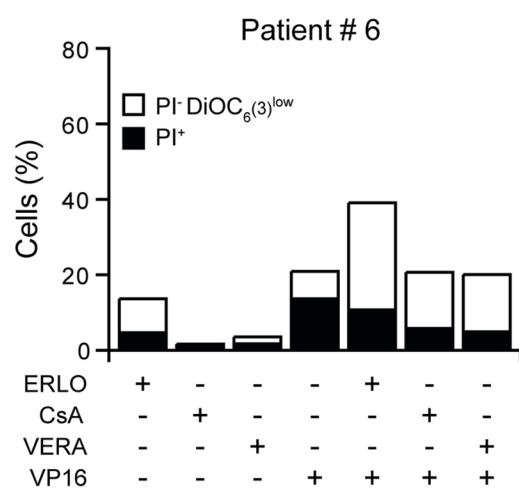
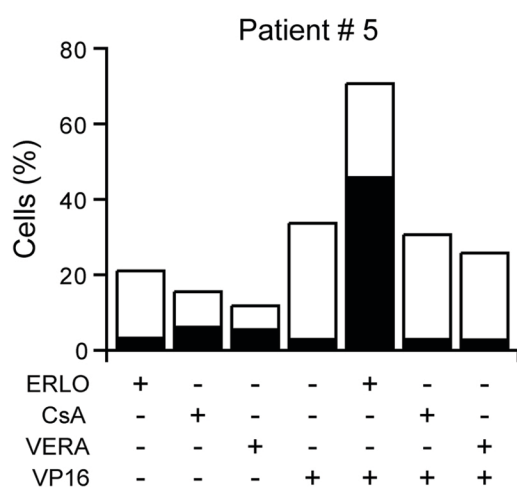
**<http://www.landesbioscience.com/journals/cc/article/22382>**



Suppl. Fig. 1



Suppl. Fig. 2



Suppl. Fig. 3

***Supplementary Figure 1. Gefitinib increases etoposide cytotoxicity in an additive fashion.***

KG-1 cells were left untreated or incubated with the indicated concentrations of gefitinib (GEFI) alone or combined with 0.1-2  $\mu$ M etoposide (VP16) for 48 h, followed by colorimetric determinations of cell viability/proliferation. Combination index (CI) values as calculated - according to the Harbron's method - for the indicated concentrations of GEFI as well as for the global dataset are reported (means  $\pm$  95% confidence interval).

***Supplementary Figure 2. Inhibitors of SRC kinases, PI-3K and mTOR fail to affect drug***

***efflux via P-gp and MRPs but downregulate P-gp exposure on the cell surface.*** KG-1 cells were kept in control conditions (DMSO) or administered with 10  $\mu$ M erlotinib (ERLO), 10  $\mu$ M PP2, 10  $\mu$ M LY294002 (LY), 10 nM rapamycin (RAPA), 10 nM calcein, 20 nM DiOC<sub>2</sub>(3), 1  $\mu$ M cyclosporine A (CsA) or 10  $\mu$ M MK-571, alone or in combination, for 48 h and then subjected to cytofluorometry for the assessment of P-glycoprotein (P-gp) exposure on the cell surface (**A,B**), of for 2 h and then analyzed for DiOC<sub>2</sub>(3) (**C,D**) or calcein retention (**E,F**). Panels **A**, **C** and **E** report representative fluorescence profiles. In panels **B**, **D** and **F**, quantitative data are reported (P-gp fluorescence normalized to that of the isotype control, calcein and DiOC<sub>2</sub>(3) fluorescence normalized to that of control conditions, means  $\pm$  SEM, n = 3). \*\* $p$ <0.01, \*\*\* $p$ <0.001, ns = non significant (ANOVA plus Dunnett's test), as compared to DMSO-treated cells (**B**), to cells loaded with DiOC<sub>2</sub>(3) only (**D**), or to cells loaded with calcein only (**F**). ISO, isotype control; MFI, mean fluorescence intensity; RFI, relative fluorescence intensity.

***Supplementary Figure 3. CsA and verapamil are less efficient than erlotinib in increasing the cytotoxic potential of etoposide in patient-derived CD34<sup>+</sup> cells.***

Patient-derived CD34<sup>+</sup> cells were incubated with 5  $\mu$ M erlotinib (ERLO), 1  $\mu$ M cyclosporine A (CsA), 10  $\mu$ M verapamil (VERA) and 0.5  $\mu$ M etoposide (VP16), alone or in combination, for 48 h, then

subjected to cytofluorometry for the quantification of cell death-associated parameters. Quantitative data on the percentage of cells exhibiting mitochondrial transmembrane potential dissipation ( $\text{PI}^- \text{DiOC}_6(3)^{\text{low}}$ ) or the breakdown of plasma membrane ( $\text{PI}^+$ ) are reported for one representative experiment ( $n = 1$ ).